N-Acylvanillamides: Development of an Expeditious Synthesis and Discovery of New Acyl Templates for Powerful Activation of the Vanilloid Receptor

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A simple and general synthesis of vanillamides was developed and employed to screen acids from the fatty and isoprenoid pools for new acyl templates of biological relevance as capsaicin analogues. Potent activation of the human vanilloid receptor 1 (VR1) was observed for the vanillamides of certain polyfunctional acids from both pools, showing that the vanilloid activity of capsaicinoids can be substantially improved by introducing polar groups and/or unsaturations on the acyl moiety. The activity of the unsaturated analogues was maintained or even increased by cyclopropanation, while ω dimerization led to a substantial increase of activity. Because of the wide structural diversity of the library of compounds screened, these observations could not be translated into a single framework of structure−activity relationships. Nevertheless, a series of new highly active leads was identified, validating the pharmacological potential of the unnatural combination of natural building blocks to provide new bioactive compounds.

Introduction

N-Acylvanillamines (*N*-AVAM), a class of compounds unique to the genus *Capsicum*, are exemplified by capsaicin (CPS, **1a**),[1] the major pungent principle of hot pepper and the archetypal vanilloid.[2] *N*-AVAM have been at the center of intense research activity aimed at elucidating the basis of their antinociceptive properties and exploiting their therapeutic potential. These studies provided evidence of definite structure−activity relationships; however, the potency of the parent compound could not be increased significantly, and no better clinical lead emerged from these investigations.[3] This, and the discovery of alternative templates with improved potency and/or pharmacological profiles (resiniferonoids,[4a] *N*-vanilly-*N*′-(3-acyloxy-2-benzylpropyl)-thioureas,[4b] *N*-alkylhomovanillamides,[4c] and *N*-vanillyl-*N*′-benzylureas[4d]), substantially shifted the focus of vanilloid research from *N*-AVAM.

Modification of the amide region and the vanillyl moiety of CPS has been pursued in a systematic and extensive way,[3] but information on the acyl moiety is still scanty. This element has a high degree of conformational freedom because six torsion angles can be varied in the range of 0−360°, and the resulting rotameric distribution spans a wide range of geometries, with hundreds of low-energy conformations.[5] Many of these might not be appropriate for binding and, thus, are essentially inactive, but a systematic conformational searching with constrained probes of CPS would be prohibitive because of the number of torsional variables involved. Furthermore, certain observations suggest that the lipophilic domain of the capsaicin receptor has element(s) capable of binding polar groups or, alternatively, that certain structural elements can preorganize, in conformational terms, the lipophilic acyl moiety of *N*-AVAM for binding. Thus, while long-chain analogues of CPS like *N*-stearoylvanillamine (**1b**) are inactive, the introduction of unsaturations fully restores activity, as cogently demonstrated by *N*-oleylvanillamine (olvanil, **1c**).[6] Both alternatives hint at the possibility of substantially increasing the activity of CPS by suitable modification of the acyl chain, but this opportunity has remained, surprisingly, largely untested.

Long lipophilic sequences resembling the acyl moiety of CPS but constrained in a covalent or conformational way and occasionally interrupted by polar groups (hydroxyl, ketone carbonyl, double bond(s)) are present in a host of compounds from the natural products pool, mostly fatty and terpenoid acids. Many of them are easily available and could provide interesting probes for characterizing the lipophilic domain of the capsaicin binding site. These compounds might also expand the pharmacological profile of *N*-AVAM as shown by the peculiar activity of vanillamides derived from polyunsaturated fatty acids, like *N*-arachidonoylvanillamine (arvanil, **1d**).[7] This hybrid between the vanilloid CPS and the endocannabinoid anandamide can activate vanilloid and cannabinoid receptors more powerfully than related pure agonists of either receptors,[7] a finding which places *N*-AVAM in a much broader perspective and validates them as interesting tools for targeting multiple receptors involved in pain perception and inflammation.

These observations provided a strong rationale for screening the natural products pool for new acyl templates of biological significance as *N*-AVAM constitu-

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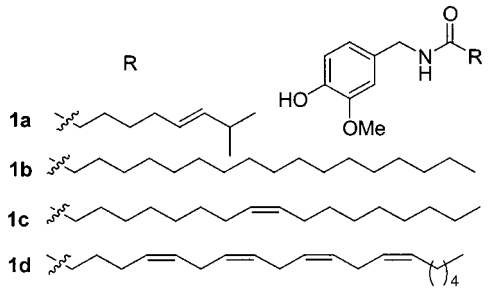


ents. To this aim, we have developed a new and expeditious procedure for the synthesis of these compounds and report its application to the discovery of several new and highly active templates from both the terpenoid and fatty acid series.

Chemistry

Despite the pharmacological relevance of vanillamides, there is still a need for a simple and mild method to prepare these compounds, especially in a single step and with an easy isolation procedure. The major problem is the chemoselectivity of the acylation. In situ activation with carbodiimide condensing agents (DCC, EDC) requires protection of the phenolic hydroxyl of vanillamine[8] because discrimination between the amino group and the phenolic hydroxyl is poor.[9] *N*,*O*-Diacyl derivatives formed in the reaction can be selectively O-deacylated with pyrrolidine,[10] but this strategy is untenable with expensive acids, with one full equivalent being wasted, and problems can arise with base-labile functionalities. Protection of vanillamine is also necessary when the acid is activated ex situ as a hydroxysuccinimide derivative or as a mixed anhydride.[8] Acylation under Schotten−Baumann conditions shows excellent selectivity for N- versus O-acylation;[6] however, activation of the acid as a chloride is required, and formation of emulsions can take place with long-chain acids. Furthermore, while formation of acyl chlorides is a routine operation for simple unfunctionalized acids, carefully controlled conditions are required with polyunsaturated acids, and protection on the acyl moiety is necessary for hydroxy acids.

To overcome these limitations, we have developed a protocol for acylation of vanillamine via mixed phosphoric anhydrides. Acyl activation with the commercially available reagents diethyl phosphorocyanidate (DEPC)[11] and propane phosphonic acid anhydride (PPAA)[12] could be attained in situ under mild conditions, and the mixed phosphoric anhydrides obtained in this way displayed the same excellent selectivity of acyl chlorides for N- versus O-(phenolic) acylation. Formation of mixed anhydrides from carboxylic acids was faster than phosphorylation of the nucleophilic sites of vanillamine, and a reduced reactivity toward alcoholic hydroxyls was also observed, thus making hydroxyl protection on both vanillamine and hydroxy acids unnecessary. This reactivity pattern, and the possibility of generating vanillamine in situ from its air-stable hydrochloride, eventually reduced the whole process into a one-pot and experimentally simple operation, while the formation of emulsions could be prevented by using a dry workup, an option impossible under Schotten−Baumann conditions. The mixed phosphoric anhydride protocol could also be applied successfully to the preparation of other bioactive phenolic amides (tyramides, dopamides, and *p*-hydroxyanilides).[13]

Table 1. Amidation Yields and Biological Evaluation of the Vanillamides **1a**−**4b**[a]

compound	amidation procedure (yield)[b]	percentage of maximum response	pEC_{50}
1a (capsaicin)		65.4 ± 4.6	7.7 ± 0.1
1b	A (76%)	32.4 ± 2.3	N M[c]
1c (olvanil)	A (81%), B (77%)	67.2 ± 7.1	9.3 ± 0.2
1d (arvanil)	A (95%), B (79%)	74.8 ± 3.5	9.3 ± 0.2
1e	A (78%)	70.9 ± 3.2	7.4 ± 0.1
1f (farvanil)	A (73%)	79.9 ± 1.2	9.3 ± 0.3
1g	A (80%)	85.0 ± 4.8	8.7 ± 0.1
1h	A (78%)	85.0 ± 5.1	6.7 ± 0.3
1i	A (56%)	74.5 ± 1.0	6.1 ± 0.1
1j (retvanil)	A (98%), B (86%)	92.6 ± 8.2	8.4 ± 0.1
1k	B (88%)	34.6 ± 1.5	N M
1l	A (85%)	91.4 ± 5.3	8.5 ± 0.1
1m	B (75%)	73.7 ± 2.2	7.9 ± 0.1
1n	A (86%)	70.7 ± 0.3	8.9 ± 0.1
1o (rinvanil)	B (84%)	85.0 ± 4.9	8.5 ± 0.1
1p	A (90%), B (91%)	85.0 ± 4.1	8.7 ± 0.1
1q	B (85%)	93.5 ± 5.9	9.4 ± 0.5
1r	A (82%)	69.7 ± 0.9	8.3 ± 0.2
1s	A (80%)	67.0 ± 1.2	8.2 ± 0.1
1t	*a*[d]	86.7 ± 3.2	8.9 ± 0.1
1u	*a*	98.0 ± 7.1	8.3 ± 0.2
1v	*a*	91.0 ± 4.9	7.1 ± 0.3
1w	*a*	96.2 ± 6.9	8.5 ± 0.1
1x	*a*	91.2 ± 4.4	8.6 ± 0.1
2a	A (68%)	37.5 ± 2.5	N M
2b	A (31%)	14.3 ± 2.3	N M
2c	A (80%), B (7%)	4.0 ± 3.0	N M
2d	B (50%)	5.8 ± 2.2	N M
2e	A (36%)	88.6 ± 4.5	6.9 ± 0.1
2f	A (91%)	78.9 ± 3.7	6.7 ± 0.1
3a	B (46%)	63.6 ± 4.2	6.1 ± 0.1
3b	A (71%)	59.3 ± 5.1	7.7 ± 0.1
3c	*b*[e]	83.4 ± 8.9	6.3 ± 0.1
3d	*b*	85.7 ± 5.3	8.8 ± 0.1
4a	A (58%)	16.4 ± 2.5	N M
4b	A (52%)	37.2 ± 3.1	N M

[a] Data are means ± SEM of three separate determinations. The maximum response was measured at 10 μM for the less-potent compounds and at 1 μM for the most-potent ones. The doses tested were 0.1, 1, 10, 100, and 1000 nM for the most-potent compounds and 0.1, 1, and 10 μM for the less-potent compounds. [b] A: DEPC protocol. B: PPAA protocol. [c] N M = non measurable. [d] Cyclopropanation yield from the corresponding monounsaturated VA: **1t**, 45%; **1u**, 36%; **1v**, 43%; **1w**, 40%; and **1x**, 42%. [e] Yield from **1p**: 21% for **3c**; 25% for **3d**.

With simple, saturated fatty acids, amidation of vanillamine via mixed phosphoric anhydrides gave yields comparable to those obtained with acyl chlorides, but the milder reaction conditions dramatically increased yields with polyenoic acids (Table 1). Thus, the reaction between arachidonic acid and vanillamine hydrochloride in the presence of DEPC and triethylamine gave arvanil (**1d**) in 95% yield. The yield with PPAA was lower (79%) but still considerably higher compared to that observed when arachidonoyl chloride was reacted with vanillamine hydrochloride under Schotten−Baumann conditions (34%).[6] Similarly, the vanillamide of retinoic acid (**1j**) could be prepared in essentially quantitative yield, and ximeninic acid, an unstable enine, could also be amidated in satisfactory yield to **1n** (63%). The mixed anhydride protocol could also be extended successfully to the preparation of the vanillamides of hydroxy (**1o**, **1r**, **2b**−**f**) and bicarboxylic acids (**4a**,**b**). Compared to DEPC, PPAA has several

desirable attributes with respect to cost, toxicity, and environmental factors, but its inherent lower reactivity made it unsuitable with hindered acids, such as those from the triterpenoid series. These could be amidated, generally in rather low yield, only under DEPC promotion (Table 1, entries **2b**−**e**). Remarkably, reaction of these acids with vanillamine using peptide coupling reagents (e.g., BOP) failed completely, an observation which underscores the synthetic potential of the mixed anhydride protocol.

Monounsaturated *N*-AVAM were cyclopropanated using a diethylzinc-based Simmons−Smith protocol,[14] while the vanillamide of eicosandioic acid (**3c**) was prepared from the vanillamide of undecylenic acid (**1p**) by homodimerization via cross metathesis[15] and hydrogenation.

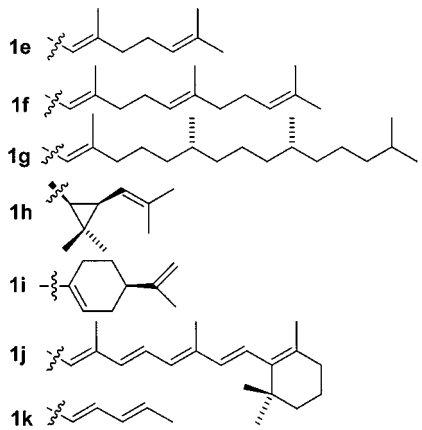
Biological Evaluation

The activity of *N*-AVAM on the CPS binding sites of vanilloid receptors was evaluated by measuring the entry of Ca^{2+} into human embryonic kidney HEK-293 cells overexpressing the human VR1,[16] a typical VR1-mediated effect. In this assay, CPS induces a dose-dependent increase of intracellular calcium (pEC_{50} = 7.7) which is not observed in wild-type HEK-293 cells and is blocked by the vanilloid receptor antagonist capsazepine.[17] This method allows the combined evaluation of *potency* (as pEC_{50}, where EC_{50} is the molar concentration necessary to induce a relative half-maximal response for each compound) and *efficacy* (as a percent of the maximum possible absolute effect obtained with ionomycin). These two parameters reflect the affinity of a compound for the CPS binding site on VR1 and its capacity to induce a conformational change eventually translating into a functional response (i.e., calcium efflux), respectively. Although compound solubility and permeability through the cell membrane can affect efficacy and potency, respectively, at least the former of these factors was kept under control by testing the compounds in both methanol and dimethylsulfoxide (DMSO). This did not lead to any observed significant differences in efficacy, except for some of the polycyclic di- and triterpenoid derivatives which were more efficacious in DMSO. This method is not biased by the possible heterogenicity of native vanilloid receptors, a typical drawback of the rodent preparations used in the structure−activity studies carried out before the cloning of VR1.[4,6,8,10] Furthermore, this method can be carried out under accurately defined conditions where parameters that normally influence the VR1-mediated calcium response, such as cell number, degree of confluency, presence of carrier protein, etc., can be tightly controlled (Experimental Section). Finally, to demonstrate unequivocally the involvement of VR1 receptors in their pharmacological actions, the most-potent *N*-AVAM in this assay, that is, farvanil, rinvanil, and acetyl-rinvanil, were also tested in untransfected wild-type HEK-293 cells and were found to be inactive on Ca^{2+} influx.

Results and Discussion

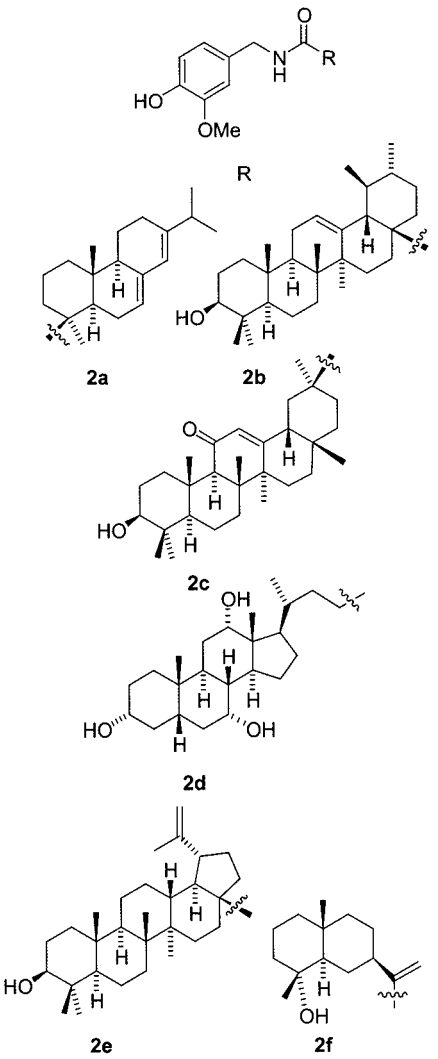
Most in vitro studies of vanilloid activity were carried out on rat dorsal root ganglia where a heterogeneous population of binding sites for CPS exists;[2] therefore, it was interesting to revisit and expand the structure−activity relationships that emerged in these studies employing the cloned human vanilloid receptor and new acyl analogues. The development of an expeditious protocol for acylating vanillamine was central to our program, and in situ activation via mixed phosphoric anhydrides proved to be successful in solving the chemoselectivity of the reaction and avoiding unproductive protection/deprotection steps. An amidation protocol based on commercially available and cheap reagents (PPAA or DEPC) was optimized using unstable polyunsaturated acids (arachidonic and ximeninic acids) as a probe and was then extended to a series of substrates from the isoprenoid and fatty acid pools (Table 1).

The choice of the acids was inspired by the possibility of evaluating conformational and covalent constraints within the lipophilic domain of the acyl moiety of CPS. This structural element has a high degree of conformational freedom, allowing the attainment of self-coiled conformations.[5] Instead, linear isoprenoid residues show a strong tendency to adopt an extended conformation,[18] a bias which is essentially solvent-insensitive,[18] while their lipophilic terpenoid skeleton can be covalently constrained in a variety of cyclic frameworks. In the event, replacement of the isodecenoic acyl residue of CPS with geranic acid had little effect on activity (Table 1) despite the presence of further unsaturation and the different connectivity of the C-10 chain (cf. **1a** and **1e**). Homologation to farnesic acid caused a marked increase in activity, affording "farvanil" (**1f**), one of the two most-potent compounds observed in this study. Potency was not increased further by additional prenylogation to phytoic acid, a C-20 compound (cf. **1g**). Reshaping of the C-10 chain into a cyclopropane ring, as in the vanillamide of chrysanthemic acid (**1h**), decreased the potency as compared to that of geranylvanillamine (**1e**) and so did covalent constraint of the isoprenoid residue into a cyclohexane ring, as exemplified by the vanillamide of (*S*)-perillic acid (**1i**). Polyunsaturation within the isoprenoid moiety had a dramatic effect on activity because the vanillamide of retinoic acid (retvanil, **1j**) was more efficacious as well as 1 order of magnitude more potent than CPS. Polyunsaturation alone was not sufficient to impart vanilloid properties as shown by the inactivity of the VA of sorbic acid (**1k**), an observation suggesting that allylic methyl groups play an important role in the interaction of linear lipophilic moieties with VR1.

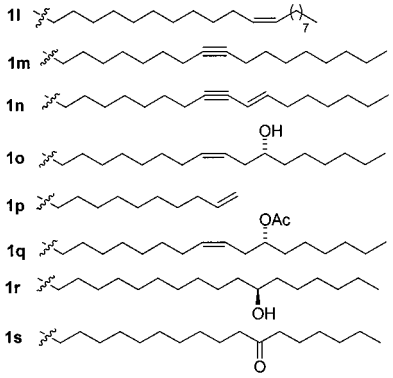


Constraint of the CPS acyl moiety into a polycyclic structure gave puzzling results. Thus, the vanillamide of abietic acid was almost inactive (**2a**), as were those of ursolic, 18β-glycyrrhetic, and cholic acids (**2b**−**d**, respectively). Nevertheless, the vanillamide of betulinic

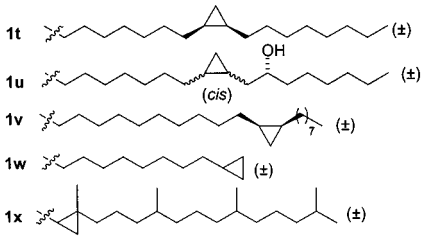
acid (**2e**), a lupane derivative, and that of ilicic acid (**2f**), a bicyclic sesquiterpene, were quite efficacious, albeit 1 order of magnitude less potent than CPS.



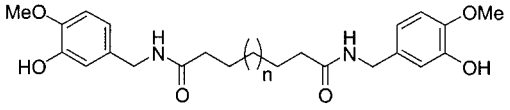
Next, we investigated the activity of the vanillamides of a series of fatty acids. Previous studies had established that the vanilloid activity of linear fatty acids peaks at C-9 and then fades with a further increase of the chain length.[6] Within C-18 acids, a double bond at C-9 was shown to fully restore activity.[6] The archetypal vanillamide of fatty acids is olvanil (**1b**).[6] Its efficacy, but not its potency, could be increased by extension of the side chain (VA of erucic acid, **1l**), an effect that cannot be observed by replacement of the double bond with a triple bond (VA of stearolic acid, **1m**) or an enine moiety (VA of ximeninc acid, **1n**) (Table 1). Introduction of an allylic hydroxyl on the distal homoallylic position (VA of ricinoleic acid, **1o**) and truncation of the acyl moiety at the distal olefin terminus (VA of undecylenic acid, **1p**) also increased the efficacy without greatly altering potency. The vanillamide of ricinoleic acid (rinvanil, **1o**) had potency comparable to that of retvanil (**1j**),[19] a finding that suggests the presence of polar elements within the apolar pocket accommodating the acyl residue of CPS. However, this polar element(s) does not establish a hydrogen bond with the hydroxyl group of rinvanil because the activity of the latter was significantly increased, rather than reduced, by acetylation (**1q**), while hydrogenation of the double bond, as in **1r** and its corresponding ketone **1s**, reduced efficacy but not potency. Taken together, these observations suggest that a second pocket, capable of interacting with ester groups, might also be present in the apolar cleft accommodating the acyl residue of *N*-AVAM.



With the aim of dissecting the conformational and polar contributions of unsaturations to the activity of unsaturated fatty acid amides, a selection of monounsaturated acids were cyclopropanated. With all compounds tested (cyclopropanated VA of oleic (**1t**), ricinoleic (**1u**), erucic (**1v**), and undecylenic (**1w**) acids) an increase of efficacy, but not of potency, was observed (Table 1), supporting a conformational role for the double bond of long-chain fatty acids. The increase in efficacy induced by cyclopropanation was especially marked for the vanillamide of undecylenic acid (**1w**). Cyclopropanation also mildly increased the activity of phytoic acid vanillamide, a C-20 monounsaturated linear terpenoid acid (cf. **1g** and **1x**).

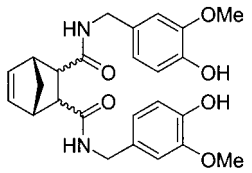


Because the VR is a multimeric receptor,[2] we finally investigated a series of dimeric vanillamides obtained either by the bidirectional amidation of various dicarboxylic acids or by the dimerizative metathesis of ω-unsaturated acids. The VA of both azelaic acid (**3a**) and sebacic acid (**3b**) showed activity lower or comparable to that of CPS, but increasing the chain length led to a remarkable increase of efficacy, as exemplified by the bis-vanillamide **3c** (Table 1).



	n
3a	3
3b	4
3c	14
3d	14 $\Delta^{11}(E+Z)$

Indeed, the olefinic precursor of **3c** (compound **3d**) was also higly active, while the bis-vanillamides of *exo-cis*- and *trans*-norbornene dicarboxylic acids (**4a** and **4b**, respectively) were almost inactive, showing that a long, and possibly monounsaturated, aliphatic tether is necessary for vanilloid activity of bicarboxylic acids.



4a *cis (endo)*
4b *trans* (±)

The results achieved from evaluating a series of isoprenoid and fatty acids can be summarized as follows:

(1) Linear isoprenoic acids are excellent replacements for the isodecenoyl moiety of CPS, and reshaping of the isoprenoid residue to accommodate a monocyclic system is tolerated. The polyunsaturated sesquiterpenoids farnesic acid and retinoic acid afforded the most-potent and efficacious vanillamides, respectively (farvanil, **1f** and retvanil, **1j**). The substantial inactivity of the VA of sorbic acid (**1k**) points to an important role for chain length and the presence of methyl branchings.

(2) The VA of polycyclic di- and triterpenoid acids were almost inactive with the notable exception of the VA of betulinic acid (**2e**), a remarkable observation because the VA of a closely related compound (ursolic acid, **2b**) was almost inactive.

(3) Within C-18 fatty acids, the substantial inactivity on the VA of stearic acid (**1b**), previously established using an in vivo assay,[6] was also confirmed at the receptor level. On the other hand, vanilloid activity could be induced by midchain unsaturation, as in olvanil (**1c**), and was tolerant to elongation (VA or erucic acid, **1l**) and to the presence of an oxygen function on the distal side of the double bond, as in rinvanil (**1o**). Cyclopropanation of olvanil (**1c**) and other monounsaturated vanillamides (**1o**, **1l**, and **1p**) led to increased efficacy, suggesting that the double bond essentially has a conformational effect, preorganizing the long chain for bonding. Nevertheless, the increased efficacy and/or potency of the oxygenated olvanil analogues rinvanil (**1o**) and acetylrinvanil (**1q**) is a strong indication that the lipophilic binding site of vanilloids also has elements capable of interacting with a polar function, as confirmed by the activity of the non-olefinic analogues of rinvanil **1r** and **1s**.

(4) ω-Dimerization of capsaicinoids leads to increased activity, provided that the alkyl tether linking the two vanillyl moieties is long enough, as in **3c** and **3d**. This might be due to the simultaneous activation of two of the four monomers that constitute the quaternary structure of VR1 in expression systems,[20] an interaction requiring a definite distance between the vanillyl moieties.

Conclusions

Certain acids from the isoprenoid and unsaturated fatty acid pools can provide highly active vanillamides, whose activity on VR1 is comparable to that of other vanilloid prototypes,[4b−d] including resiniferonoids.[4a] In particular, the VA of farnesic, retinoic, and ricinoleic acids (farvanil, retvanil, and rinvanil; **1f**, **1j**, and **1o**, respectively) as well as acety-rinvanil (**1q**) qualify as novel and powerful vanilloid leads, worth an in-depth investigation of their structure−activity relationships. Like olvanil (**1c**)[6] and arvanil (**1d**),[7] these compounds can be viewed as "unnatural natural products",[21] the result of the artificial combination of natural building blocks (vanillamine and fatty or isoprenoid acids) into unnatural assemblies. Their interesting biomedical profile validates the still largely untapped potential of the expansion of the natural products pool by the artificial combinations of natural building blocks and/or the triggering of reactivity patterns apparently overlooked by Nature.[22]

Experimental Section

Materials. Commercially available reagents and solvents were used without further purification. CH_2Cl_2 was dried by distillation from CaH_2, and THF was dried by distillation from sodium benzophenone. Silica gel 60 (70−230 mesh, Macherey-Nagel) was used for open column chromatography (CC). Unless stated otherwise, the carboxylic acids used in this investigation are commercially available in satisfactory purity (>95%) and were purchased from Fluka-Aldrich or Alexis Biochemicals and used as such. Commercial ricinoleic acid (ca. 80%, Fluka) was purified by CC (hexanes/EtOAc gradient, from 9:1 to 8:2). Farnesic,[23] phytoic,[24] perillic,[25] dehydroricinoleic,[26] and dihydrodehydroricinoleic[26] acids were prepared from the corresponding commercially available alcohols or aldehydes according to literature. Chrysanthemic acid was prepared by the hydrolysis (15 equiv of LiOH, 2:1 ratio of THF/water) of the corresponding commercially available ethyl ester. Ximeninic, ursolic, 18β-glycyrrhetic, betulinic, and abietic acids were a generous gift from Indena, Milano, Italy. Ilicic acid was available from previous phytochemical work.[27]

Biological Assays. Overexpression of human VR1 cDNA into human embryonic kidney HEK-293 cells was carried out as described previously.[16] Cells were grown as monolayers in a minimum essential medium supplemented with nonessential amino acids, 10% fetal calf serum, and 0.2 mM glutamine, and were maintained under 95%/5% O_2/CO_2 at 37 °C. The effect of the substances on $[Ca^{2+}]_I$ was determined by using Fluo-3, a selective intracellular fluorescent probe for Ca^{2+}.[7b] One day prior to the experiments, almost confluent (70%) cells were transferred into six-well dishes coated with poly-L-lysine (Sigma) and grown in the culture medium mentioned above. On the day of the experiment, the cells (50−60000 per well) were loaded for 2 h at 25 °C with 4 μM Fluo-3 methyl ester (molecular probes) in DMSO containing 0.04% Pleuronic. After the cells were loaded, the cells were washed with Tyrode (pH 7.4), trypsinized, resuspended in Tyrode, and transferred into the cuvette of the fluorescence detector (Perkin-Elmer LS50B) under continuous stirring. The fact that fluorescence was measured in a quartz cuvette allowed us to conduct experiments in the absence of bovine serum albumin, which inhibits the calcium response of *N*-AVAM as determined in previous studies. Experiments were carried out by measuring cell fluorescence at 25 °C (λ_{EX} = 488 nm, λ_{EM} = 540 nm) before and after the addition of the test compounds at various concentrations. Potency data are expressed as the −log of the concentration exerting a half-maximal effect (pEC_{50}), whereas the efficacy of the effect was determined by comparing it to the analogous effect observed with ionomycin (4 μM).

Acylation of Vanillamine. (A) DEPC Protocol. Synthesis of arvanil (**1d**) as exemplificative. To a solution of high-purity arachidonic acid (>98%, 262 mg, 0.86 mmol, Alexis Biochemicals) in dry THF (2.5 mL) were added vanillamine hydrochloride (196 mg, 1.03 mmol, 1.2 molar equiv), DEPC (216 mg, 198 μL, 1.3 mmol, 1.5 molar equiv), and triethylamine (309 μL, 224 mg, 2.06 mmol, 2.4 molar equiv). The solution was stirred under nitrogen for 1.5 h. Volatiles were removed

under reduced pressure, and the residue was adsorbed on silica gel 60 (ca. 1 g) and purified by open column chromatography (5 g silica gel, 8:2 ratio of hexanes/EtOAc as eluant), affording 360 mg (95%) of arvanil (**1d**) as a colorless oil. **(B) PPAA Protocol.** Synthesis of rinvanil (**1o**) as exemplificative. To a cooled (0 °C) solution of ricinoleic acid (500 mg, 1.67 mmol) in dry CH_2Cl_2 (5 mL) were added vanillamide hydrochloride (318 mg, 1.67 mmol, 1 molar equiv), triethylamine (950 μL, 690 mg, 3.3 mmol, 2 molar equiv), and PPAA (50% EtOAc solution, 1.2 mL, 3.3 mmol, 2 molar equiv). After the mixture was stirred for 15 min at 0 °C, the ice bath was removed, and the reaction mixture was allowed to warm to room temperature in ca. 1 h. Volatiles were removed under reduced pressure, and the residue was adsorbed on silica gel 60 (ca. 2 g) and purified by open column chromatography (15 g silica gel, 3:7 ratio of petroleum ether/EtOAc as eluant) to afford 509 mg (70%) of rinvanil (**1o**) as a colorless oil.

Cyclopropanation of Monounsaturated Vanillamides. Synthesis of **1t** as exemplificative. To a solution of olvanil (**1c**, 200 mg, 0.48 mmol) in dry benzene (20 mL) were added Et_2-Zn (1 M in THF, 7.2 mL, 7.2 mmol, 15 molar equiv) and diiodomethane (0.58 mL, 7.2 mmol, 15 molar equiv) dropwise at room temperature. The reaction mixture was then heated at 65 °C for 6 h and worked up by cooling, acidification with 2 N H_2SO_4, and extraction with EtOAc. The organic phase was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. The residue was purified by CC (10 g silica gel, petroleum ether/EtOAc gradient). Fractions eluted with a 6:4 ratio of petroleum ether/EtOAc afforded 94 mg (45%) of **1t** as a colorless oil.

Dimerizative Metathesis of Undecylenyl Vanillamide. To a solution of **1p** (310 mg, 0.97 mmol) in dry CH_2Cl_2 (3.5 mL) was added Grubbs catalyst (8 mg, 0.0097 mmol, 0.1 molar equiv). The reaction mixture was stirred under nitrogen at room temperature for 3 days, and then worked up by evaporation and chromatography (8 g silica gel, petroleum ether/EtOAc gradient). Fractions eluted with a 2:8 ratio of petroleum ether/EtOAc gave 100 mg (25%) of **3d** as a powder.

Catalytic Hydrogenation of the Dimer 3d. The crude, unchromatographed residue from the dimerizative metathesis of the vanillamide of undecylenic acid (310 mg) was dissolved in MeOH (3 mL), and 10% Pd(C) (10 mg) was added. The suspension was stirred for 3 h under a hydrogen atmosphere (balloon), and then worked up by filtration. The residue was purified by CC (8 mL silica gel, 1:9 ratio of petroleum ether/EtOAc as eluant) to afford 85 mg of **3c** (21% from **1p**) as an off-white powder.

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Supporting Information Available: General methods and characterization (mp, IR, MS, and ^{1}H and ^{13}C NMR data) of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Posting. This manuscript was released ASAP on 7/9/2002 with minor errors in the structures of **1d** and **1k**. The correct version was posted on 8/8/2002.

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